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Comparative Evaluation of Carbonated Beverage and Calcium Glycerophosphate Supplemented Carbonated Beverage on Surface Microhardness of Enamel and Restorative Glass Ionomer Cement-An Invitro Study

Dr. T. Hemalatha[1*]; Dr. R. Krishnakumar[2]; Dr. S. Syed Shaheed Ahamed[3]; Dr. S. Lokesh[4]
[1*]Postgraduate, [2]Professor, [3]Associate Professor, [4]Assistant Professor
[1,2,3,4]Department of Paediatric dentistry, Government Dental College and Hospital, Cuddalore district, Annamalai Nagar, Chidambaram

Corresponding Author: **Dr. T. Hemalatha**

Abstract
Background: Erosion caused by carbonated beverages can reduce the surface microhardness of enamel and restorative materials. Adding calcium glycerophosphate (CaGP) to carbonated beverages may help mitigate these effects. **Aims:** To evaluate the effect of carbonated beverages with and without calcium glycerophosphate on the surface microhardness of enamel and glass ionomer cement (GIC). **Materials and Methods:** Twenty enamel blocks (2×3×4 mm) prepared from 10 extracted premolars and 20 GIC pellets (10 mm diameter, 2 mm height) were analysed. Initial surface microhardness was measured using a Vickers microhardness tester. Samples were divided into four groups: Group I: Enamel in carbonated beverage. Group II: Enamel in carbonated beverage with CaGP. Group III: GIC in carbonated beverage. Group IV: GIC in carbonated beverage with CaGP. Samples underwent immersion in solutions five times daily for 10 minutes, stored in artificial saliva between cycles, for seven days. Post-immersion microhardness was measured. **Statistical analysis used:** Paired and independent t-tests were applied. **Results:** Significant reduction in surface microhardness occurred in Groups I and III ($p<0.001$). Groups II and IV, with CaGP, showed no significant reduction. **Conclusion:** Adding 10mM CaGP to carbonated beverages effectively minimized surface microhardness loss in enamel and GIC.

Key-words: Carbonated beverage, Erosion, Calcium glycerophosphate, Enamel, Glass ionomer cement, Surface microhardness

Introduction

In recent years, dietary habits have shifted significantly, with a notable global surge in carbonated beverage (CB) consumption, especially among children and adolescents [1]. This increase is due to CBs' flavour, extensive media promotion, and their affordability and accessibility. The acidic nature of CBs, with a pH as low as 2.5, significantly contributes to enamel demineralization [2]. Their acidity arises from the formation of carbonic acid (from added CO_2) and the presence of phosphoric, citric, and tartaric acids [3]. These beverages are highly acidogenic and cariogenic due to their high sugar or artificial sweetener content [4].

Among CBs, cola is one of the most widely consumed flavours globally, containing a mix of carbonated water, sugar or sweeteners, caramel (for colour), and phosphoric acid, achieving a balanced sweet-sour flavour profile. Caffeine is typically included, and certain additives contribute to cola's distinctive taste. Despite the acidity of CBs, the high sugar content masks the sour taste [5]. Frequent consumption of acidic CBs is linked to dental erosion, as described by Pindborg, characterized by the irreversible chemical dissolution of tooth structure from non-bacterial acid sources [6]. Dental erosion begins with sensitivity, progressing to enamel wear, and is primarily caused by acids from dietary and environmental sources, with CBs being a significant contributor [7].

The erosive potential of CBs depends on factors like chelation properties, frequency, and duration of exposure. Additional influences include dietary habits, lifestyle, and biological factors such as saliva composition, flow rate, and buffering capacity [8].**Devlin et al.** reported a decrease in enamel microhardness after cola exposure, attributed to phosphoric acid's erosive effects, making cola an important study subject due to its widespread consumption and health impacts [9].

Tooth-coloured restorative materials are essential for dental restorations in children and adolescents, who are major CB consumers. Glass ionomer cement (GIC), introduced in 1969 by Wilson and Kent, is a popular tooth-coloured restorative material [10]. GIC chemically bonds with tooth structures and serves as a fluoride reservoir, beneficial for patients at high caries risk. Conventional GIC (cGIC) primarily consists of a water-soluble polymeric acid (polyalkenoic acid), calcium fluoro-alumino-silicate glass powder, and water. Setting occurs through an acid-base reaction, initially forming a gel-like silicon oxide matrix that incorporates unreacted glass particles, reinforcing the material's structure [10,11].

In the oral cavity, restorative materials face challenges like temperature changes and acidic conditions [12]. Durability depends on their resistance to chemical breakdown, as acidic environments weaken GIC by attacking its surface, gradually reducing hardness [13]. This study aims to evaluate the surface microhardness of GIC and enamel after exposure to cola, a common acidic beverage.

Preventive approaches to mitigate CB effects include fortifying drinks with calcium, phosphate, and fluoride [1].**Lussi et al**. demonstrated that adding these ions to beverages reduces erosion by saturating the solution, thereby lowering enamel solubility and enhancing its protective properties [14]. Calcium glycerophosphate (CaGP), a glycerophosphoric acid salt, has shown promise in this context. First studied by Bowen in 1972 for its anticaries potential, CaGP is FDA-approved as a food additive and recognized for its buffering capacity. It has been added to toothpaste for cariostatic effects by buffering pH, increasing calcium and phosphate in plaque, and directly interacting with enamel [1,15].

Studies indicate CaGP's potential to minimize enamel dissolution[16] and its supplementation in CBs or incorporation into restorative materials may provide broader preventive benefits. This study investigates the effect of CaGP-supplemented cola on the surface microhardness of both human enamel and GIC.

Surface microhardness testing, a reliable method for detecting changes in mineral content after acid exposure[17], is employed here to compare the effects of regular cola and CaGP-supplemented cola on enamel and GIC. This research could support the development of preventive strategies against acid-induced damage in patients frequently consuming acidic beverages.

Methodology:

The present in vitro study was undertaken in the Department of Paediatric and Preventive Dentistry, Government Dental College and Hospital, Cuddalore District, in association with the Department of Biochemistry, Government Medical College and Hospital, Cuddalore District and the Department of Manufacturing Engineering, Annamalai Universityafterobtaining approval from Institutional Review Board (approval no: GDCHIS2021PG01PEDO).

Ten premolar teeth extracted for orthodontic purposes were collected, disinfected with formalin as per CDC guidelines, rinsed, and stored in deionized water. Teeth with caries, cracks, or restorations were excluded. Two enamel blocks measuring 2x3x4 mm were cut from each premolar, yielding 20 blocks, and mounted onto acrylic bases. Twenty Type II GIC pellets (10 mm diameter, 2 mm height) were prepared with a brass mould, ensuring smooth surfaces using mylar strips and compression by glass slab. Baseline microhardness of enamel and GIC samples was measured using a Vickers hardness tester, applying a 100 g load for 15 seconds(Figure 1). A10 mM concentration of calcium glycerophosphate was preferred for addition to Cola beverage based on studies by **Barbosa et al.** and **Manaswini et al**., which demonstrated a pH increase at this concentration without adverse effects. Artificial saliva was prepared following **Sato et al.**, with specific chemical concentrations [1]. The pH of Cola was initially 2.69, rising to 5.24 with CaGP addition.

Samples were divided into four groups
Group I(n=10): Enamel blocks immersed in Cola beverage
Group II(n=10): Enamel blocks immersed in Cola beverage supplemented with CaGP
Group III(n=10): GIC pellets immersed in Cola beverage.
Group IV(n=10): GIC pellets immersed in Cola beverage supplemented with CaGP

The samples were subjected to five immersion cycles, each lasting for 10 minutes, evenly distributed over a 12-hour time period in a day at room temperature for 7 days.

Between the immersion cycles, the samples of each group were transferred and stored in artificial saliva to simulate oral environment. For every immersion cycle, Cola beverage and artificial saliva used for immersing the samples were replaced. This was in accordance with **Bajwa et al**[13].

Assessment of Post-Immersion Microhardness

The post-immersion surface microhardness of all samples (enamel blocks and GIC pellets) was assessed using a Vickers microhardness tester, applying a load of 100g for 15 seconds. Indentations were made at three different locations on each sample and the mean microhardness was determined.

Results:

The collected values were entered in MS Office Excel 2019. Statistical analysis was done using IBM SPSS Statistics for Windows, Version 23, Chicago, USA. Significance level was set at p value <0.05.

- For intragroup comparison of mean surface microhardness before and after immersion in cola beverages, paired t-tests were applied to each of the four groups: Group I, Group II, Group III, and Group IV.
- For intergroup comparison between Group I and Group II, as well as between Group III and Group IV, independent sample t-tests were applied.

Surface Microhardness of Enamel:

Group I (immersed in cola) showed a significant decrease from 299.63 HV to 279.20 HV (p=0.0001). Group II (cola + CaGP) showed minimal change, from 298.36 HV to 297.12 HV (not significant). The reduction in Group I was significantly greater than in Group II (p=0.0001).

Surface Microhardness of GIC :

Group III (immersed in cola) showed a significant decrease from 45.3 HV to 31.3 HV (p=0.0001). Group IV (cola + CaGP) showed a minor decrease from 44.7 HV to 43.9 HV (not significant). Group III's reduction was significantly greater than Group IV (p=0.0001).

Discussion:

Soft drinks consumption has increased significantly in recent years, particularly among children and adolescents. **Grimm et al**. observed several factors influencing consumption among children, such as parental and peer influence, exposure to television advertisements, a strong preference for taste, and the accessibility of soft drinks in both home and school environments [18].**Damle et al.** found children prefer cola over milk or juice, likely due to its taste and caffeine [19]. Thus, cola was selected in this study to assess its erosive effects.

Lussi et al. and Bajwa et al. reported that soft drinks contribute to erosion of dental tissues and restorations [7,13]. **Schlueter et al.** found that acidic drink intake increases erosion risk, affecting 30-50% of primary teeth and 20-45% of permanent teeth across age groups [20].

The erosive potential of carbonated beverages is influenced by pH, acidity, buffer capacity, and acid type [21]. Phosphoric acid in cola drinks releases hydrogen ions (H^+), which bind with calcium in enamel, causing mineral loss, weakening enamel, and potentially leading to hypersensitivity or pulp exposure [2,8].

The pH of beverages is identified as the most crucial determinant of erosive potential, with studies highlighting a pH below 3 as extremely erosive [22].In the present study, the cola beverage showed a pH value of 2.69 which is considered highly acidic as surface enamel demineralization begins when the pH falls below 5.5 (critical pH) [9].

The erosive impact of cola beverages on enamel is demonstrated through the chemical equation: $Ca_{10}(PO_4)_6(OH)_2 + 8H^+ \rightarrow 10Ca^{2+} + 6HPO_4^{2-} + 2H_2O$.This rate of hydroxyapatite dissolution could be reduced if calcium and phosphate ions are incorporated to the acidic beverages [21]. **Hills and Sullivan** demonstrated that saturating the demineralizing medium with calcium and phosphate effectively prevented enamel dissolution [23]. Studies have shown that incorporating food additives and dietary supplements containing calcium and phosphates, such as calcium lactate pentahydrate, sodium polyphosphates, and calcium glycerophosphate into acidic beverages resulted in a decrease in their erosive potential when compared to the unmodified beverages [24,25,26].

In the present study, calcium glycerophosphate was added to the cola beverage. The usage of Calcium Glycerophosphate (CaGP) adheres to the guidelines set by the "Food Chemicals Codex", an international reference for assessing the quality and purity of food chemicals.**Barbosa et al. and Manaswini et al.** have illustrated that the supplementation of CaGP to carbonated beverage prevents the enamel mineral loss [1,26].

The in vivo studies conducted by **Bowen, Grenby et al**. have confirmed the anticaries efficacy of CaGP[16,27].**Zaze et al**. demonstrated that incorporating 0.25% Calcium Glycerophosphate (CaGP) into low-fluoride toothpaste (500 µg F/g) produced a similar reduction in enamel demineralization compared to high-fluoride toothpaste (1100 µg F/g) [28].

According to **Lynch et al**., the mechanism of Calcium Glycerophosphate (CaGP) involves increasing calcium and phosphate concentrations in plaque which may aid in the remineralization of dentin and enamel; buffers the pH of the plaque, assisting in the preservation of a neutral or slightly alkaline environment that is less favourable to demineralization and acidogenic bacteria; may have direct interactions with the hard tissues of the teeth, creating a barrier that keeps the dentin and enamel from further demineralization. CaGP strengthens enamel and raises the phosphorus content of plaque [29].

Similar to the erosion process affecting dental hard tissues, the exposure of restorative materials to acidic challenges in the oral cavity results in their degradation and a decrease in surface microhardness, compromising their clinical performance [12]. GIC, made up of calcium fluoroaluminosilicate glass powder as its base combined with a water-soluble polymer (polyacrylic acid) is well known for its fluoride release[11].GIC gradually releases fluoride (F), aluminium (Al), silica (Si), and calcium (Ca) ions when subjected to acidic conditions in the oral environment [30]. The hydrogen ions (H^+) in the acidic environment, causes disruption of the Si-O-Si glass bond present on the surface of the glass particles. This leads to the dissolution of filler particles, resulting in the formation of numerous porous patches on the cement surface. The process of dissolution in acidic environment is more pronounced with conventional GICs, thereby leading to a reduction in the surface microhardness [11].

Thus, the present study aims to analyse the erosive effect of cola beverage on the surface microhardness of enamel and conventional glass ionomer cement, as well as to investigate the protective effect of calcium glycerophosphate incorporated into cola beverage on the surface microhardness of both enamel and glass ionomer cement.

A 10mM concentration of calcium glycerophosphate was selected based on the insights from **Barbosa et al.** and **Manaswini et al.** [1,26]. Their studies demonstrated a substantial pH increase at this specific concentration, ensuring it remains well below the threshold for causing any adverse effects during regular consumption. The addition of CaGP to the cola beverage resulted in a pH rise from 2.69 to 5.24, similar to the findings reported by **Barbosa et al.** (2.7-5.29) and **Manaswini et al.** (2.8-5.5). Moreover, the ability of CaGP to buffer acidic conditions was supported by findings from **Lynch and Ten Cate (2006)**, confirming its role in elevating pH levels.[31] This effective pH modulation of CaGP was further demonstrated by **Torsakul et al. (2023),** who observed an increase in pH levels in acidic solutions containing CaGP [32].

In this study, enamel and GIC samples were immersed five times daily over 12 hours, reflecting medium intake frequency [13,33]. A 7-day immersion period was chosen, as significant changes in physical properties typically occur within this timeframe in acidic solutions, consistent with **Bajwa et al. (2016)**

To closely simulate the oral environment and replicate clinical scenarios, artificial saliva was selected as the preferred storage medium. This is consistent with the methodology adopted by **Manaswini et al.** and **Bajwa et al.** in their respective studies[13].

Vickers microhardness test was used to evaluate the effect of carbonated beverage on surface microhardness of enamel and glass ionomer cement (GIC). **Kodaka et al. (1992)** explored the relationship between mineral content and microhardness of enamel, emphasizing microhardness' role in predicting enamel's resistance to acid attacks [34].**Yu et al.** (2009) used Vickers microhardness to assess changes in the hardness of enamel and restorative materials after acidic and abrasive challenges [35].**Gutiérrez-Salazar and Reyes-Gasga** recommended the Vickers indenter for its ability to detect small surface changes [36]. A force of 100 g was applied for 15 seconds, similar to the study by **Bajwa et al.** At this force, the development of cracks on the surface of the material was prevented [13].Consequently the surface microhardness was measured using the size of indentation on the samples.

The present study showed a significant decrease in the surface microhardness of enamel samples immersed in cola beverage (Group I) from 299.63 HV to 279.20 HV (p=0.0001) (Table 1). In Group II, cola with CaGP resulted in a minor reduction from 298.36 HV to 297.12 HV, likely due to CaGP's calcium and phosphate content reducing mineral loss by saturating the beverage with respect to hydroxyapatite [1,37]. This aligns with findings by **Manaswini et al.** and **Scaramucci et al.,** who observed improved enamel microhardness with calcium-enriched beverages [1,24].Furthermorestudies incorporating CPP-ACP into acidic drinks prevented erosion of enamel due to increase in pH and the increased levels of calcium and phosphate ions at the surface of enamel [38,39]. Studies conducted by **Lussi et al** and **Wonghantee et al**. indicated that calcium present in yoghurt prevented the loss of enamel surface microhardness, aligning with the results of the present study [37,40].

Furthermore, **Barbosa et al.** demonstrated a decrease in bovine enamel erosion when carbonated beverages were supplemented with CaGP, leading to reduced enamel wear [26]. Thus the ability of calcium glycerophosphate (CaGP) to influence the pH of beverages and provide buffering ions plays a pivotal role in preventing erosion by carbonated drinks. The covalent bonding of phosphate and calcium to glycerol in CaGP prevents these components from reacting within the beverages. This is crucial as phosphate ions, by binding to protons in the carbonated beverage, elevate their pH, ensuring the availability of free calcium ions to prevent enamel from erosion. Additionally, the incorporation of CaGP reduces the loss of calcium and phosphate in direct proportion to the mineral concentration of enamel, effectively preventing mineral loss and maintaining surface microhardness [1].

Similar to that of the enamel samples, GIC samples immersed in cola beverage (Group III) (Table 2) demonstrated a significant reduction in surface microhardness, from 45.3 HV to 31.3 HV (p=0.0001). This observation corresponds with the findings of **Bajwa et al. (2016)**, which showed significant decrease in microhardness of GIC following exposure to carbonated beverage [13].

GIC samples immersed in cola beverage supplemented with CaGP (Group IV) showed a minor decline in surface microhardness from 44.7 HV to 43.9 HV. This diminished reduction of surface microhardness of glass ionomer cement in Group IV could be associated with the calcium and phosphate present in CaGP, which prevented the dissolution of GIC. Similar findings were reported by **Wongkhantee S et al. (2006)**, where there was an increase in microhardness of glass ionomer cement after immersion in yoghurt which could be attributed to the calcium and phosphate availability at the surface of the cement [37]. Similarly,**Nadia et al.** observed that the applying CPP-ACP to Glass ionomer cement resulted in decreased reduction of GIC microhardness, which was attributed to the buffering action of CPP-ACP and its role as a calcium reservoir [41].

Thus, the addition of Calcium Glycerophosphate to carbonated beverage, with its buffering action and role as a reservoir of calcium and phosphate, significantly contributes to the prevention of enamel and glass ionomer cement (GIC) microhardness, enhancing their resistance to erosive challenges. There are certain limitations in the present in-vitro study as there are some in vivo variables that cannot be duplicated in-vitro. Beverages in the oral cavity can stimulate saliva production, which helps to prevent erosion. Moreover, salivary phosphatases may release phosphate ions, potentially improving the effectiveness of CaGP compared to in vitro conditions.

Conclusion:

From the results obtained in the present in vitro study, the following conclusions can be inferred.

- The carbonated beverage without CaGP demonstrated a more pronounced reduction in surface microhardness for both enamel and GIC.
- The addition of CaGP to the carbonated beverage significantly reduced the decrease in surface microhardness of both enamel and glass ionomer cement.
- Incorporating CaGP into the carbonated beverage resulted in a significant increase in the pH of the beverage.
- The supplementation of a 10 mM concentration of CaGP to carbonated beverage presents a practical approach for mitigating their erosive impact on both dental enamel and restorative GIC.

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Table 1: Intragroup comparison of mean surface microhardness of Enamel and GIC before and after immersion in carbonated beverages (cola beverage and cola beverage supplemented with CaGP)

Groups	Baseline Mean±SD (HV)	Post immersion Mean ±SD(HV)	t test	p value
Group I (n=10)	299.63±6.8	279.20±10.9	5.6	0.0001*
Group II (n=10)	298.36±5.08	297.12±7.4	0.48	0.637
Group III(n=10)	45.3±1.8	31.3±1.7	17.7	0.0001*
Group IV(n=10)	44.7±2.3	43.9±3.1	0.5	0.58

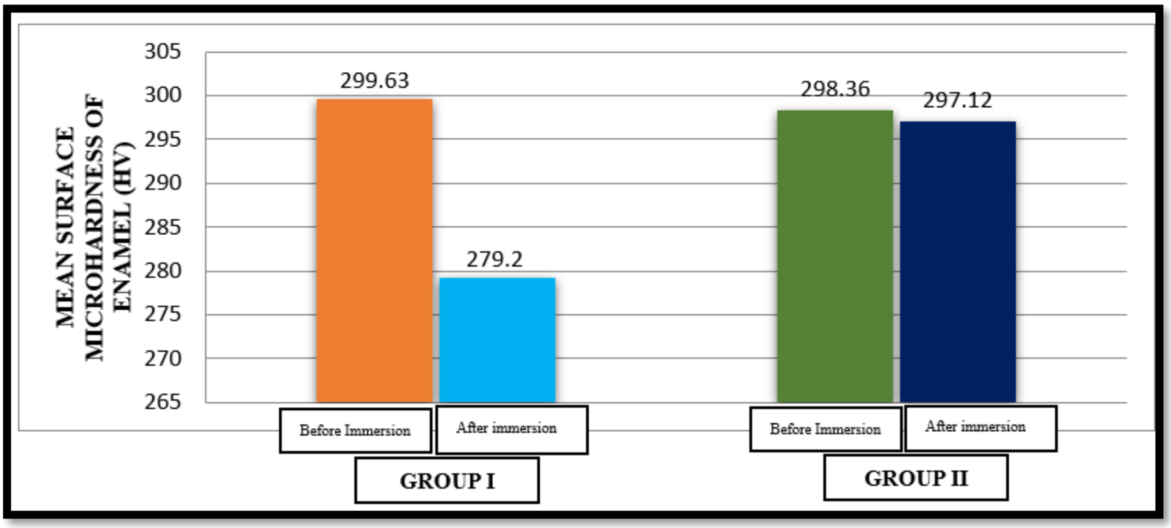
Paired t test; *Statistically significant

Table 2: Intergroup comparison of post-immersion mean surface microhardness of Enamel and GIC (immersed in cola beverage and cola beverage supplemented with CaGP)

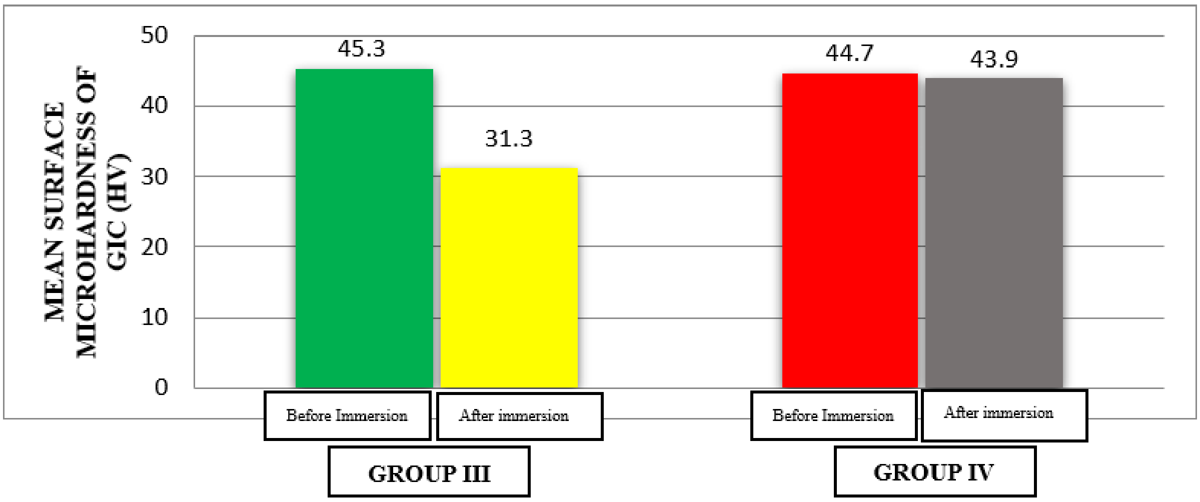
Groups	Post immersion Mean ± SD(HV)	Mean difference	t test	p value
Group I (n=10)	279.2±10.9	-17.9	-4.27	0.0001*
Group II (n=10)	297.1±7.4			
Group III (n=10)	31.3±1.7	-12.8	-10.8	0.0001*
Group IV (n=10)	43.9±3.1			

Independent sample t test;* Statistically significant

Graph 1: Intragroup comparison of mean surface microhardness of Enamel before and after immersion in carbonated beverages (cola beverage and cola beverage supplemented with CaGP)



Graph 2: Intragroup comparison of mean surface microhardness of GIC before and after immersion in carbonated beverages (cola beverage and cola beverage supplemented with CaGP)



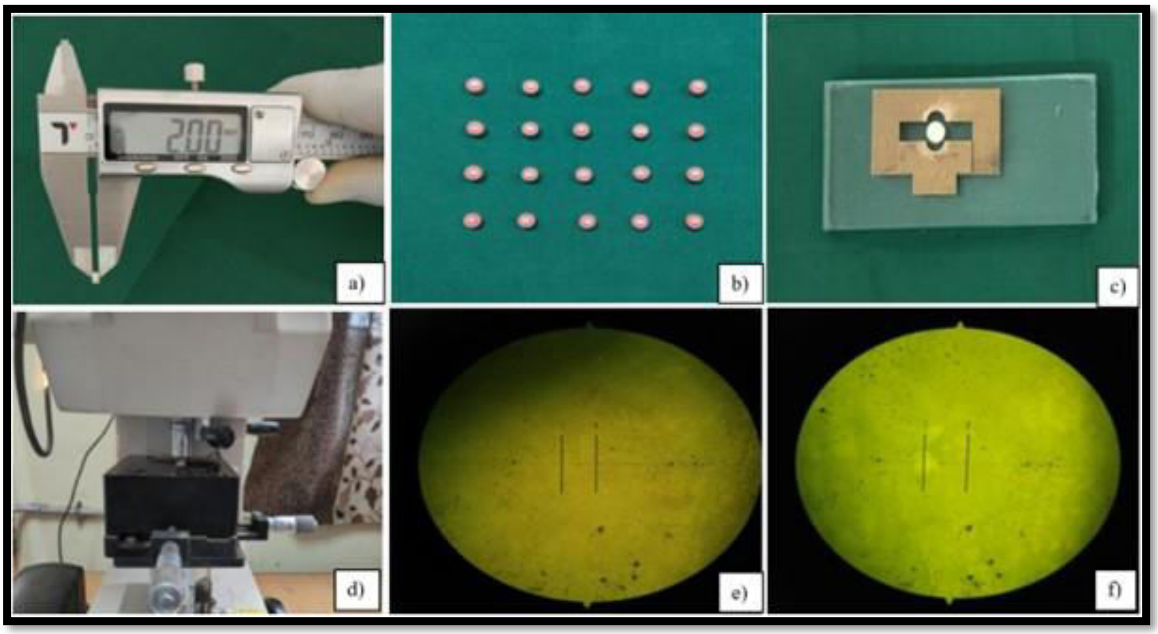


Figure1

a) Enamel block measured using Vernier caliper b)Enamel blocks mounted on acrylic

c) Retrieval of GIC sample from the brass mould d) Testing of microhardness e),f)Indentation measured on Enamel and GIC samples.